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SHORT COMMUNICATIONS

A Comparative Study of the Effect of the Lectins of *Azospirillum brasilense* Sp7 and Its Mutant on the Activity of Some Enzymes in Plant Cells

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Our earlier studies showed that the soil plant-associated nitrogen-fixing bacterium *Azospirillum brasilense* Sp7 contains a surface glycoprotein (lectin) that is specific to L-fucose (1.87 mM) and D-galactose (20 mM) [1]. The most efficient way to study the function of bacterial surface structures is to use mutants defective in the synthesis of particular components of these structures. However, we did not succeed in obtaining bacterial cells that were unable to synthesize the lectin. On the other hand, we did succeed in obtaining a mutant strain whose cells lost the ability to aggregate erythrocytes and to interact with lectin-specific antibodies but retained surface molecules with a molecular mass and carbohydrate specificity identical to those of the lectin of the parent strain [2].

An understanding of the molecular mechanisms underlying bacterial–plant interactions requires knowledge of the functional role of the lectins of the soil bacteria that form nitrogen-fixing symbioses in the plant rhizosphere. The lectins of azospirilla may perform the role of adhesins that provide for the specific attachment of bacterial cells to wheat roots [3]. Other functions of these lectins are unknown. The aim of this work was to study the ability of the lectins of azospirilla to influence the activity of the hydrolytic enzymes of plant cells.

The soil plant-associated bacterium *A. brasilense* Sp7 was obtained from the Winogradsky Institute of Microbiology, Russian Academy of Sciences, Moscow. The mutant strain *A. brasilense* Sp7.2.3, defective in lectin activity, was derived from strain Sp7 by transposon mutagenesis [2]. Surface lectins were obtained

The effect of the lectins of *A. brasilense* Sp7 and its mutant Sp7.2.3 on the activity of the hydrolytic enzymes of wheat seedling roots

Experimental variant	*A. brasilense* Sp7			*A. brasilense* Sp7.2.3		
	α-Glucosidase, U/mg	β-Glucosidase, U/mg	β-Galactosidase, U/mg	α-Glucosidase, U/mg	β-Glucosidase, U/mg	β-Galactosidase, U/mg
F_e	4.0 ± 0.2	2.2 ± 0.2	2.0 ± 0.5	4.0 ± 0.2	2.2 ± 0.2	2.0 ± 0.5
F_e + L	50.0 ± 0.8	57.0 ± 0.8	51.4 ± 1.2	32.5 ± 1.2	29.0 ± 1.1	22.0 ± 2.5
F_e + (L + F)	5.3 ± 0.1	4.0 ± 0.1	10.2 ± 0.4	20.0 ± 1.4	17.0 ± 0.9	6.0 ± 1.0
F_e + (L + G)	4.9 ± 0.2	4.1 ± 0.2	12.0 ± 0.2	12.0 ± 0.5	2.0 ± 0.4	2.4 ± 0.4
F_m	14.5 ± 1.4	6.2 ± 0.1	6.0 ± 0.6	14.5 ± 1.4	6.2 ± 0.1	6.0 ± 0.6
F_m + L	47.0 ± 1.2	74 ± 2.5	42.7 ± 2.7	20.0 ± 1.2	20.0 ± 0.5	16.0 ± 1.6
F_m + (L + F)	19.3 ± 1.0	21.0 ± 0.3	30.0 ± 1.2	17.5 ± 0.4	19.5 ± 0.8	12.2 ± 0.4
F_m + (L + G)	18.0 ± 0.2	21.0 ± 2.1	25.0 ± 1.4	19.0 ± 1.7	20.0 ± 1.6	11.9 ± 2.5
F_a	29.2 ± 1.7	2.0 ± 0.2	4.0 ± 0.2	29.2 ± 1.7	2.0 ± 0.2	4.0 ± 0.2
F_a + L	60.4 ± 0.9	31.9 ± 1.1	24.0 ± 1.8	39.8 ± 0.7	19.0 ± 1.4	13.0 ± 1.2
F_a + (L + F)	32.0 ± 1.2	12.0 ± 0.5	6.0 ± 0.4	28.3 ± 1.2	16.5 ± 1.4	12.5 ± 0.5
F_a + (L + G)	29.3 ± 0.8	10.0 ± 0.4	4.9 ± 0.2	29.0 ± 0.5	17.0 ± 0.5	12.0 ± 0.5

Note: F_e, fraction of root exometabolites; F_m, fraction of root membranes; F_a, fraction of root apoplasts; F + L, fraction incubated with lectin; F + (L + F), fraction incubated with lectin preincubated with L-fucose; F + (L + G), fraction incubated with lectin preincubated with D-galactose.

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from bacterial cells as described earlier [4]. Three fractions of plant tissues (the root exocomponents, membranes, and apoplasts) were prepared from the roots of 4-day-old wheat cv. Saratovskaya 29 seedlings grown aseptically from surface-sterilized grains [3, 5]. The fractions were incubated with a lectin solution. The optimal values of lectin concentration and incubation time were chosen experimentally. α-Glucosidase, β-glucosidase, and β-galactosidase were assayed by the amount of nitrophenol produced enzymatically from 4-nitrophenyl-α-D-glucopyranoside, 4-nitrophenyl-β-D-glucopyranoside, and 4-nitrophenyl-β-D-galactopyranoside, respectively [6]. The amount of nitrophenol produced was measured spectrophotometrically at λ = 425 nm. One unit of enzymatic activity was defined as the amount of enzyme that converted 1 nmol of substrate in 1 min. Specific enzymatic activities were expressed per milligram protein. Experimental data were processed by using Student's *t*-test [7].

The incubation of the *A. brasilense* Sp7 lectin at a concentration of 40 μg/ml with the fractions of the root exocomponents, membranes, and apoplasts for 1 h augmented the activity of the measured plant enzymes as compared to the control (the activity of these enzymes in the fractions of the wheat roots that were not incubated with the lectin) (see table). The lectin of the mutant strain taken at the same concentration exerted a slightly weaker effect. The maximum activity of all the enzymes was observed in the fraction of the root exometabolites. The pretreatment of both lectins (from the parent and mutant strains) with the specific haptens L-fucose and D-galactose diminished (but did not abolish) their effect on the enzyme activities. The effects exerted by the haptens were similar in all cases except for the experimental variant with the lectin of the mutant strain and the fraction of the root exometabolites, in which D-galactose was more active than L-fucose. In the case of the root exometabolites, the specificity of the lectin of the parent strain was maximum with α- and β-glucosidases and that of the lectin of the mutant strain was maximum with β-galactosidase. At the same time, no differences were observed for the interaction of both lectins with the α- and β-glucosidases of the root membranes and apoplasts, although these lectins exhibited differences in their interaction with the β-galactosidases of the two root fractions.

Thus, the interaction of the lectin of the mutant strain is the most specific with the β-galactosidase of the membrane fraction, whereas the lectin of the parent strain exhibited the maximum specificity with the root apoplasts. These differences may indicate the existence of different mechanisms of lectin–enzyme interactions.

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